

prepared and the frequencies of dicentrics and rings are estimated in first division metaphases or micronuclei in binucleate cells. The points of the dose-response relationship are fitted to an equation which is linear-quadratic for low LET (linear energy transfer) radiation and linear for high LET radiation.

The correct fitting procedure is not trivial because it requires an appropriate weighting of data points. Several laboratories have produced their own curve fitting programs for internal use but these are frequently not user-friendly and not available to outside users. Therefore, a PC-based freely available program called CABAS, for fitting dose-response curves to chromosomal aberration or micronucleus data and for calculating the dose and confidence limit (CL) has been developed and tested. The program consists of (i) the main curve-fitting and dose estimating module, (ii) a module for calculating the dose in cases of partial body exposure, (iii) a module for estimating the minimum number of cells necessary to detect a given dose of radia-

tion, and (iv) a module for calculating the dose in the case of a fractionated or protracted exposure (Fig.).

The program can be downloaded as freeware from <http://www.pu.kielce.pl/ibiol/cabas> or obtained from any of the present authors. The use of the program is straightforward and it can be expected that its use will improve the precision of dose estimates by biological dosimetry in cases of radiation accidents. Furthermore, it should facilitate setting up inter-laboratory dose effect curves.

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References

- [1]. Cytogenetic analysis for radiation dose assessment. A manual. IAEA, Vienna 2001.
- [2]. Voisin P., Barquinero J.F., Blakely B., Lindholm C., Lloyd D., Lucioni C., Miller S., Palitti F., Prasanna P.G., Stephan G., Thierens H., Turai I., Wilkinson D., Wojcik A.: *Cell. Mol. Biol.*, **48**, 501-504 (2002).

THE TEMPERATURE EFFECT ON THE FREQUENCY OF RADIATION-INDUCED MICRONUCLEI IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES IS ABOLISHED BY DMSO

Kinga Brzozowska, Andrzej Wójcik

The impact of temperature on the frequency of radiation-induced chromosome aberrations in the human lymphocytes was first described by Bajerska and Liniecki [1]. We have previously described [2] experiments carried out to analyze the impact of blood temperature at irradiation *in vitro* on the level of radiation-induced micronuclei. We found that temperature of pre-irradiation incubation as well as that during exposure exerted an effect on the frequency of radiation-induced micronuclei in human peripheral blood lymphocytes. The highest frequency of micronuclei was observed for blood samples incubated at 37°C before and during irradiation *in vitro* with doses of 2 and 2.7 Gy, intermediate – at 20°C and the lowest one – at 0°C.

Blood samples were drawn from two healthy male donors aged 24 and 45 years and irradiated at 0, 20 and 37°C with X-rays 200 kVp, 5 mA, 3 mm Cu filter. The doses were: 0, 1 and 2 Gy for the first donor, and 0, 1.35 and 2.7 Gy for the second donor. For 20 min before irradiation as well as during irradiation, the blood samples were incubated at 0, 20 or 37°C. dimethylsulphoxide (DMSO) was added 5 min before irradiation at the concentration of 0.5 mol/dm³. After irradiation, the samples were centrifuged in order to discard supernatants containing DMSO; cells were then transferred into 4.5 ml RPMI 1640 medium supplemented with 25% calf serum, 2.5% phytohaemagglutinin (PHA), antibiotic solution and incubated for 72 h at 37°C and 5% CO₂. Lymphocyte preparations for micronuclei analysis were prepared according to the standard method of Fenech [3].

Whereas temperature of pre-irradiation incubation as well as that during exposure exerted a distinct effect on the frequency of radiation-induced micronuclei in human peripheral blood lymphocytes, the presence of DMSO completely abolished this effect: the dose-effect curves for all three temperatures were identical.

In the interaction of ionizing radiation with DNA one discerns direct and indirect effect, the latter produced in the water layer surrounding the DNA molecule. Since DMSO is a scavenger of the OH[•] radical which is produced in the process of water radiolysis, the presented results indicate that temperature conditions affect the indirectly induced damage. At this stage of our research, we cannot explain which mechanisms are responsible for the effect of temperature on the level of radiation-induced cytogenetic damage, although we presume that chromatin conformation may play a role. It is plausible to assume that accessibility of DNA to the radical attack depends on the steric relations of the chromatin components and specifically, on the extent of protection by proteins. Temperature dependent structural transitions in the protein-DNA complex (e.g. [4]), as well as temperature dependence of binding of specific nuclear proteins to DNA (e.g. [5,6]) have been described but not systematically explored from the point of view of chromatin radiosensitivity.

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References

[1]. Bajerska A., Liniecki J.: *Int. J. Radiat. Biol.*, **16**, 483-493 (1969).

[2]. Brzozowska K., Wójcik A.: The effect of temperature on the frequency of radiation-induced micronuclei in human peripheral blood lymphocytes. In: INCT Annual Report 2005. Institute of Nuclear Chemistry and Technology, Warszawa 2006, pp.102-103.

[3]. Fenech M.: *Mutat. Res.*, **285**, 35-44 (1993).

[4]. Peters W.B., Edmondson S.P., Shriner J.W.: *J. Mol. Biol.*, **343**, 339-360 (2004).

[5]. Santilli G., Schwab R., Watson R., Ebert C., Aronow B.J., Sala A.: *J. Biol. Chem.*, **280**, 15628-15634 (2005).

[6]. Ferreira M.E., Hermann S., Prochasson P., Workman J.L., Berndt K.D., Wright A.P.: *J. Biol. Chem.*, **280**, 21779-21784 (2005).

VARIABLE RADIOSENSITIVITY OF CHROMOSOMES 2, 8 AND 14 IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES EXPOSED TO 480 MeV/n ^{12}C -IONS

Marta Deperas-Kaminska^{1,2}, Gennady N. Timoshenko¹, Eugene A. Krasavin¹, Andrzej Wójcik^{2,3}

¹/ Joint Institute for Nuclear Research, Dubna, Russia

²/ Świętokrzyska Academy, Kielce, Poland

³/ Institute of Nuclear Chemistry and Technology, Warszawa, Poland

For a wide variety of biological effects, radiations of high linear energy transfer (LET) have been known to have greater biological effectiveness per unit dose than those of low LET [1]. Little is known about the extent of individual variability in the radiosensitivity of human cells to high LET radiation. In all published studies dealing with individual radiosensitivity, the studied cells (lymphocytes or fibroblasts) were only exposed to low LET radiation. The purpose of this study was to investigate by FISH the distribution of radiation-induced chromosomal aberrations in chromosomes 2, 8 and 14 in lymphocytes of 3 donors.

Irradiation of blood from 3 healthy donors was performed at the Nuclotron accelerator at the Joint

tions including the complex ones were transformed into primary breaks. The break frequencies were scaled to the whole genomic frequencies. The results are presented in Fig.1. For all donors, the lowest frequency of breaks was observed in chromosome 2 and the highest in chromosome 14 in lymphocytes of donor 3, chromosome 8 of donor 2, and at the same level in chromosomes 8 and 14 of donor 1 (Fig.1A). The found break frequency is below the expected values for chromosome 2 and above the expected values for chromosomes 8 and 14. Only for chromosome 14 of donor 2 the ratio is close to unity (Fig.1B).

The lowest frequencies of exchanges were scored in chromosome 2 and the inter-donor vari-

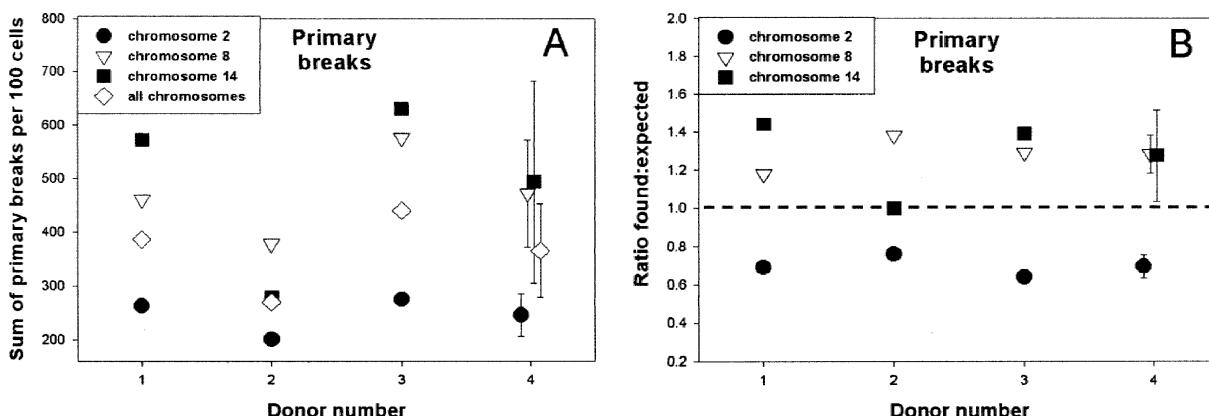


Fig.1. Primary breaks observed in the painted chromosomes of 3 donors. Breaks calculated from aberrations observed after exposed to 3.5 Gy of 480 MeV/n ^{12}C -ions were summed up and scaled to the whole genomic frequency. The absolute numbers are shown in Fig.1A and the ratios of found to expected are shown in Fig.1B. Error bars represent standard deviations from the mean; also, values are shown for the three painted chromosomes of all donors.

Institute for Nuclear Research (Dubna, Russia). Whole blood samples were irradiated with 1.1, 2.3 and 3.5 Gy of ^{12}C -ions. At the position of the sample the beam energy was 480 MeV/n and LET=10.6 keV/ μm . Chromosomes 2, 8 and 14 were painted in different colors and aberrations scored with the help of an image-analysis system, as described in [2].

In order to assess the overall radiosensitivity of the painted chromosomes, chromosomal aberra-

ability was low. Chromosomes 8 and 14 were involved in exchanges more frequently than expected on the basis of DNA content. In contrast, the involvement of chromosome 2 was less frequent than expected.

This is the first study investigating the individual radiosensitivity of chromosomes of human peripheral blood lymphocytes to heavy ions. Generally, the sensitivity of chromosome 2 was lower, and that